Patent
Attorney Docket No. 275.0003 0102

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Li *et al.*	)	Confirmation No.: 9705
Application No.: 10/038,984	)	Group Art Unit: 1635
Filed: January 4, 2002	)	Examiner: Tracy Ann Vivlemore
For: COMPOSITION AND METHOD FOR IN VIVO AND IN VITRO ATTENUATION OF GENE EXPRESSION USING DOUBLE STRANDED RNA	)	

APPELLANTS' APPEAL BRIEF

Commissioner for Patents
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Sir:

Introduction

The invention on appeal is a method for attenuating gene expression in vertebrate cells *ex vivo*. The method entails explanting a vertebrate cell from a vertebrate organism, supplying the cell with at least one double stranded RNA in a sufficient amount to attenuate expression of a target gene, and implanting the cell into a vertebrate organism.

Real Party in Interest

The real party in interest is Medical College of Georgia Research Institute , Inc. by virtue of assignment from the inventors, Yin-Xiong Li, Michael J. Farrell, and Margaret L. Kirby, recorded at Reel 013141, Frame 0502 on July 30, 2002. An exclusive license is currently held by Nucleonics, Inc.

Related Appeals and Interferences

There are no related appeals or interferences known to the appellants, their legal representatives or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

Status of Claims

Claims 1-61 were presented with the application as filed and claims 62-98 were added by amendment. Of these claims, claims 1-74, 77, and 80-81, have been cancelled. Claims 75, 76, 78, 79, and 82-98 are pending and at issue in this appeal, and are set out in an Appendix to this brief.

Status of Amendments

The appellants submitted responses dated September 20, 2006 and October 16, 2006 with amendments and arguments in response to the non-final Office Action dated April 20, 2006. Both responses were entered and considered in the Final Office Action dated December 22, 2006. The appellants filed a Notice of Appeal on June 21, 2007. An amendment after final correcting claim dependency is submitted concurrently with this brief. Entry of the amendment is expected as it pertains to matters of form and does not change the scope of the claims on appeal.

Summary of Claimed Subject Matter

The invention provides methods of attenuating expression of a target gene in a cell, particularly a vertebrate cell. See, for example, page 2, lines 20-28 and page 8, lines 7-25 of appellants' specification. Specifically, the claimed subject matter relates to attenuating target gene expression in a vertebrate cell *ex vivo* and subsequently implanting the cell into a vertebrate organism. See page 3, lines 18-25. The inventive methods comprise administering at least one double stranded RNA to the cell, wherein

the double stranded RNA is identical to at least a portion of the target gene. See appellants' specification at page 11, lines 19-31.

Although methods of inhibiting gene expression in some invertebrate and plant species using double stranded RNA have been described (see background section of appellants' specification at page 1, lines 17-29 and references cited therein), previous attempts at using similar methods in vertebrates have been unsuccessful.

To the appellants' knowledge, the methods of the present invention as defined by the pending claims are the first successful attempt at target gene silencing in explanted vertebrate cells using double stranded RNA. As described in appellants' specification (page 8, lines 8-18, page 15, line 26- page 18, line 8), these methods find various scientific and medical uses, such as methods for treating viral infections, cancer, and diseases associated with abnormal gene products as well as preventing transplant rejection.

Appellants have demonstrated the success of their method in targeting both endogenous genes and transgenes in three different types of vertebrate cells. Administration of double stranded RNA targeting the T gene in zebrafish embryos resulted in disorganized somites and truncated tails similar to the phenotype of the no tail (ntl) mutant embryos harboring a natural mutation of the T-gene (see Example 1 at page 24, line 27 to page 26, line 14). The Pax6.1 gene is thought to be involved in the development of the lens placode and the forebrain while the Nkk 2.7 gene plays an important role in heart development. Administration of double stranded RNA targeting either the Pax6.1 gene or the Nkk2.7 gene to zebrafish embryos resulted in the phenotypes expected with loss of expression of these genes (see page 29, lines 6-25 and page 29, line 27 to page 30, line 8). Furthermore, injection of GFP double stranded RNA into zebrafish embryos suppressed the transient expression of GFP in cells having a plasmid-encoded GFP gene (Example 1 at page 24, lines 2-14). These working examples show that both transgenes as well as endogenous genes can be specifically targeted and silenced in vertebrate cells using the claimed methods.

In addition to the several examples performed in zebrafish, appellants have also shown that the claimed methods can be employed to silence specific genes in other vertebrate cells. For example, the expression of HirA, a gene associated with increased persistent truncus arteriosus, was effectively suppressed by administering HirA double stranded RNA to neural crest explants from chick. See Example 2 at page 35-36 of the specification. Example 3 describes attenuation of GFP expression in mammalian (rat)[1] cell culture by administering double stranded RNA targeting the GFP transgene (see appellants' specification at page 36, line 6 to page 37, line 7). Thus, appellants have convincingly shown that the methods of the invention as described by the pending claims are effective at silencing specific target genes in vertebrate cells, including zebrafish, avian, and mammalian cells as specific working examples.

Grounds of Rejection to be Reviewed on Appeal

I. Novelty

The Examiner has finally rejected claims 75, 76, 78, 79, 82-91, and 93-98 under 35 U.S.C. 102(e) as unpatentable over U.S. Patent No. 6,506,559 to Fire *et al.* The issue can be summarized as follows:

Is the appellants' method for attenuating expression of a target gene in a vertebrate cell *ex vivo* anticipated by the disclosure of the Fire *et al.* reference?

Appellants submit that the claimed method is novel and is not anticipated by the disclosure of the cited reference for the reasons detailed herein.

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[1] Appellants' specification states that the experiments in Example 3 were performed in mouse NIH/3T3 cells. However, in the preliminary amendment dated April 28, 2003 appellants submitted a declaration by Dr. Yin-Xiong Li indicating that the cells used for the experiments in Example 3 were actually rat ROS cells and were inadvertently labeled as mouse NIH/3T3 cells (see paragraph 4 of the declaration dated April 22, 2003).

II. Non-Obviousness

The Examiner has finally rejected claims 75, 76, 78, 79, and 82-98 under 35 U.S.C. 103(a) as unpatentable over U.S. Patent No. 6,506,559 to Fire *et al.* in view of a publication by Ekenberg *et al.* (Promega Notes Magazine, 1994, Vol. 46, pgs. 14-17). Although the Examiner has listed all the pending claims as rejected on this basis, her supporting arguments are directed only at the limitation of treating the double stranded RNA with RNase as recited in claim 92. Thus, the appellants believe that the rejection under 35 U.S.C. 103 applies only to claim 92. On this basis, the issue can be stated as follows:

> Do the references, considered singly or together, make it obvious to attenuate expression of a target gene in an explanted vertebrate cell by administering double stranded RNA treated with RNase prior to delivery to the cell?

For the reasons set forth below, the appellants submit that the claimed method is <u>not</u> obvious from the cited references.

Grouping of Claims

The claims have been separated into two distinct groups for the arguments pertaining to the rejection under 35 U.S.C. 102(e): (1) claims 75, 76, 79, 82-91, and 93-98 and (2) claim 78. The patentability of each group will be argued separately and each should be considered exclusively.

With regard to the arguments in response to the rejection under 35 U.S.C. 103(a), appellants understand that the rejection applies only to claim 92.

Arguments

I. Rejection under 35 U.S.C. 102(e) over U.S. Patent No. 6,506,559

A. <u>Claims 75, 76, 79, 82-91, and 93-98</u>

The methods of the invention as claimed are directed to suppressing expression of a target gene in a vertebrate cell *ex vivo*. In particular, cells of a vertebrate organism are explanted, double stranded RNA is introduced into the explanted cells to attenuate

expression of a target gene, and the explanted cells containing the double stranded RNA are implanted back into the organism (see appellants' specification at page 16, lines 1-4). As demonstrated by the working examples detailed in the appellants' specification, the appellants have successfully achieved attenuation of target gene expression of both endogenous genes and transgenes in vertebrates cells from various preparations including vertebrate embryos, vertebrate explants, and vertebrate cell lines.

The Examiner has finally rejected claims 75, 76, 78, 79, 82-91, and 93-98 as allegedly being anticipated by U.S. Patent No. 6,506,559 to Fire *et al.* (Exhibit A). The Examiner believes that Fire *et al.* disclose a method for inhibiting gene expression using a double stranded RNA and that "the method is general" (See page 2 of Office Action dated April 20, 2006). She reiterates her arguments in the Final Office Action dated December 22, 2006 asserting that Fire *et al.* disclose that the method can be performed in vertebrates and in cells *ex vivo* (see page 2 of Final Office Action dated December 22, 2006).

The Examiner alleges that Fire *et al.* disclose a method for attenuating gene expression in vertebrates by administration of double stranded RNA and cites column 8 of U.S. Patent No. 6,506,559 as support for her argument. See page 2 of Final Office Action dated December 22, 2006. In fact, the relevant paragraphs of column 8 (lines 13-17 and lines 37-40) of Fire *et al.* merely mention that the cell containing the target gene may be derived from or contained in any organism and lists vertebrates in a laundry list of several other organisms. "The disclosure in an assertedly anticipating reference must provide an enabling disclosure of the desired subject matter; mere naming or description of the subject matter is insufficient, if it cannot be produced without undue experimentation." M.P.E.P. §2121.01. A disclosure is enabling if the public is in possession of the claimed invention prior to the date of the invention. M.P.E.P. §2121.01. "Such possession is effected if one of ordinary skill in the art could have combined the publication's description of the inventions with his [or her] own knowledge to make the claimed invention." *In re Donohue*, 766 F.2d 531, 226 USPQ 619 (Fed. Cir. 1985). Appellants submit that Fire *et al.* do **not** provide an enabling disclosure for a method of

attenuating target gene expression in a **vertebrate** cell and that at the time the invention was made, one of ordinary skill in the art could not have attenuated target gene expression in a vertebrate cell using the methods disclosed by Fire *et al.* without undue experimentation.

At the time the present invention was made, it was well known in the art that vertebrate cells possessed a non-specific response to double stranded RNA molecules. First observed in cells infected with viral RNA, double stranded RNA molecules were reported to cause several toxic events, including general translational arrest and induction of interferon synthesis. See, for example, Isaacs and Lindenmann (1957) Proc. R. Soc. Lond. (Biol) 147: 258-267 (Exhibit B) and Carter and DeClercq (1974) Science 186: 1172-1178. (Exhibit C). This non-specific response or "stress response" to double stranded RNA molecules results in the activation of the double stranded RNA-dependent protein kinase (PKR), RNAase L, and Mx pathways. PKR (previously known as DAI kinase) phosphorylates a number of substrates, including the translation initiation factor eIF2α and IκB, inducing translational arrest and apoptosis. See, for instance, Clemens and Elia (1997) J. Interferon Cytokine Res. 17: 503-524 and Proud, C.G. (1995) Trends in Biochemical Sciences 20: 241-246. (Exhibits D and E). The activation of the PKR kinase is dependent on the length of the double stranded RNA with increased activation efficiency correlated with increasing length of the double stranded RNA molecule. Double stranded RNA longer than 30 base pairs will activate PKR, while maximum activation appears to occur with RNA molecules longer than 80 base pairs. See Manche *et al.* (1992) Mol. Cell. Bio. 12: 5238-5248. (Exhibit F). In fact, even shorter dsRNA molecules are known to mediate cytotoxicity in mammalian cells. See, for example, Sioud, M. (2005) J. Mol. Bio. 348: 1079-1090 (Exhibit G) and Reynolds et al. (2006) RNA 12: 1-6 (Exhibit H).

A number of publications in the field reported difficulties in achieving attenuation of target gene expression or target gene silencing in vertebrate cells using methods employing double stranded RNA (dsRNA) molecules due to the stress response. For example, Tuschl and colleagues reported that while sequence-specific gene silencing was

effective in *Drosophila* cell lysates, similar results were not obtained in rabbit reticulocyte lysates. See Tuschl *et al.* (1999) Genes and Development 13: 3191-3197 (Exhibit I). The authors state that "[T]he addition of 10 nM of dsRNA to the rabbit reticulocyte lysate caused a profound and rapid, nonspecific decrease in mRNA stability.....The nonspecific destruction of mRNA induced by the addition of dsRNA to the rabbit reticulocyte lysate presumably reflects the previously observed activation of Rnase L by dsRNA." See Tuschl *et al.,* page 3195, left-hand column, citations omitted. The authors go on to say that "If RNAi exists in mammals, as might be predicted from the presence of RNAi-like phenomena in invertebrates, plants, and fungi,... it is likely obscured by the rapid induction by dsRNA of nonspecific antiviral responses." Tuschl *et al.*, pg. 3195.

Caplen *et al.* observed similar results when administration of dsRNA to mammalian cells from three different species failed to show a specific down-regulation of gene expression. Gene (2000) 252: 95-105 (Exhibit J). Caplen *et al.* concluded that their results "were consistent with the well-documented interferon-induced non-specific response of mammalian cells to dsRNA." See Caplen *et al.*, page 103, right-hand column. Similar problems were encountered in other vertebrate cells as illustrated by the findings of Oates and colleagues. Oates *et al.* (2000) Developmental Biology 224: 20-28 (Exhibit K). The authors reported that dsRNA injected into early zebrafish embryos produced a nonspecific depletion of several endogenous mRNAs and concluded that "RNAi appears unsuited to application in the zebrafish embryo...." (page 21, left-hand column).

Later-published papers continued to document the difficulty and inconsistent results obtained with administration of dsRNA to vertebrate cells. Caplen *et al.* (Proc. Natl. Acad. Sci. USA (2001) 98: 9742-9747, attached as Exhibit L) summarized the state of the field in 2001 by stating the following:

> As yet, clear evidence for the generality of an RNAi-like mechanism in vertebrate cells is lacking. Several studies have reported evidence for dsRNA-triggered silencing in particular certain vertebrate systems, early

> embryos of mice, zebrafish, and *Xenopus*, as well as Chinese hamster ovary cells. At the same time, numerous reports have described failures to observe gene-specific RNAi effects in different vertebrate systems, demonstrating instead nonspecific effects of dsRNA on gene expression. These nonspecific effects have not been surprising as there is an extensive literature describing a variety of nonspecific responses induced by dsRNAs in mammalian cells. (page 9742, right-hand column, citations omitted).

Elbashir *et al.* also recognized the apparent lack of RNAi mechanisms in mammalian cell culture and attributed the difficulty in identifying these mechanisms to the nonspecific interferon response induced by dsRNA molecules longer than 30 base pairs. Nature (2001) 411: 494-498 (Exhibit M). Even Fire, the first named inventor on the cited prior art patent, expressed uncertainty whether the methods of attenuating gene expression by administration of double stranded RNA in nematodes was applicable to vertebrate cells. See Montgomery and Fire (1998) Trends in Genetics 14: 255-258 (Exhibit N). He states that activation of the PKR kinase by dsRNA in mammalian cells "unleashes a vehement but somewhat non-specific response leading to general translational arrest" and speculates that "[a]ny gene-specific interference by dsRNA in PKR-proficient mammalian cells would be dependent on a transient lapse in the PKR response, or on a controlled level of dsRNA that was incapable of activating PKR" (page 258, columns 1-2).

It is apparent from the studies described above that the skilled artisan had considerable difficulty in using double stranded RNA to suppress expression of target genes in vertebrate cells despite the published findings in nematodes and plants. Moreover, considerable experimentation appeared to be required to attempt to circumvent the well-known stress response induced by double stranded RNA. Appellants note that the studies described above were published after the disclosure of the nematode data by Fire *et al.* in journal articles (i.e. Exhibit N) and the corresponding PCT publication (WO 1999/032619), suggesting that Fire's disclosure in U.S. Patent No.

6,506,559 did not enable one of ordinary skill in the art to practice the disclosed methods in vertebrate cells. In fact, the Examiner herself cited several post-Fire references in a previous enablement rejection (see pages 5-8 of Office Action dated September 8, 2004) documenting the unpredictability of methods of suppressing target gene expression in vertebrate cells by administration of dsRNA and states that "The unpredictability of attenuating/inhibiting expression of a target gene in vertebrates by RNA interference (RNAi) is evident in post-filing art" (page 5 of Office Action dated September 8, 2004). Thus, it is clear that despite the disclosure of U.S. Patent No. 6,506,559 to Fire *et al.*, one of skill in the art was unable to suppress target gene expression in vertebrate cells effectively without undue experimentation.

As noted above, the disclosure of an anticipatory reference must be enabling. "A claimed invention cannot be anticipated by a prior art reference if the allegedly anticipatory disclosures cited as prior art are not enabled." *Amgen, Inc. v. Hoechst Marion Roussel, Inc.*, 314 F.3d 1313, 1354, 65 USPQ2d 1385, 1416 (Fed. Cir. 2003). The Federal Circuit has established that in order to enable, the anticipatory reference must teach one of ordinary skill in the art to make or carry out the claimed invention without undue experimentation. *Minnesota Mining and Manufacturing Co. v. Chemque Inc.*, 64 USPQ2d 1270, 1278 (Fed. Cir. 2002); *see also Elan Pharmaceuticals Inc. v. Mayo Foundation for Medical Education and Research*, 68 USPQ2d 1373, 1376 (Fed. Cir. 2003). Although the Court has made a distinction between the enablement requirement under section 102 of the statute and that under section 112 of the statue, it is clear that an anticipatory reference must be "enabling in the sense that it describes the claimed invention sufficiently to enable a person of ordinary skill in the art to carry out the invention." *See Impax Laboratories, Inc. v. Aventis Pharmaceuticals, Inc.*, 468 F.3d 1366, 1383 (Fed. Cir. 2006).

In the instant case, Fire *et al.* fails to teach a person of ordinary skill in the art to carry out the invention in vertebrate cells without undue experimentation as evidenced by the numerous publications post-Fire that document failures to achieve target gene silencing in vertebrate cells. Undue experimentation "is not a single, simple factual

determination, but rather is a conclusion reached by weighing many factual considerations." *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Factors relevant to a determination whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance present, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. *Id* at 737. The disclosure of Fire *et al.* contains only working examples of attenuating target gene expression in nematodes, in which the stress response to double stranded RNA is absent. No examples using vertebrate cells are disclosed. The state of the art and the unpredictability of the art have been described in detail above and indicate that even after the disclosure by Fire *et al.*, the stress response in vertebrate cells presented a technical problem in attenuating target gene expression using dsRNA. Although the level of skill in the art is deemed to be high, the lack of guidance in the specification on how to administer dsRNA molecules to circumvent or overcome the stress response in vertebrate cells to achieve sequence-specific suppression of gene expression leaves the skilled artisan at a loss on how to practice the invention in vertebrate cells. In light of these factors, it is clear that one of ordinary skill in the art would not be able to carry out the invention disclosed in Fire *et al.* in vertebrate cells.

Recently, in *Impax Laboratories, Inc. v. Aventis Pharmaceuticals*, Inc., the District Court of Delaware on remand from the Federal Circuit used a similar analysis to determine that a prior art patent (the '940 patent) disclosing the compound riluzole in a list of several compounds to treat a myriad of diseases was not enabled and therefore did not anticipate claims to using riluzole to treat amyotrophic lateral sclerosis (ALS). The Court stated that "[T]he '940 patent embraces in formula I hundreds to thousands of different compounds and cites numerous medical conditions associated with the effects of glutamate. Nothing in the '940 patent directs one skilled in the art to recognize that riluzole can be used to treat ALS....In these circumstances, the Court is not persuaded that the mere mention of riluzole is sufficient to put one skilled in the art in the

possession of the claimed invention as is required to support a conclusion of enablement." *See Impax Laboratories, Inc. v. Aventis Pharmaceuticals, Inc.*, 496 F. Supp. 2d 428, 432 (D. Del. 2007) (Exhibit O). As is the case here, Fire *et al.* simply recites vertebrate cells as one of many cell types in which the target gene can reside. Fire *et al.* do not provide any guidance or suggestion on how to practice the disclosed methods in vertebrate cells that would place the skilled artisan in possession of the invention in light of the well-known stress response in vertebrate cells.

The Federal Circuit agan recently examined the issue of enablement of an anticipating reference in *Forest Laboratories Inc. v. Ivax Pharmaceuticals, Inc.*, 501 F.3d 1263, 84 USPQ2d 1099 (Fed. Cir. 2007) (Exhibit P). The Federal Circuit upheld the District Court's determination that a prior art reference (the Smith reference) did not anticipate a claim to the positive enantiomer of citalopram as the reference did not enable one of ordinary skill in the art to obtain the compound. *Id.* at 1268. The Smith reference disclosed numerous enantiomers of particular drugs and their effects on serotonin uptake in brain tissue. The reference mentions that racemic citalopram is also of interest. The court found that the method of resolving the racemic mixture of citalopram (chiral high performance liquid chromatography or HPLC) was new and unpredictable at the time of the invention and that several people in the field, including Smith, failed to resolve the enantiomers of citalopram using chiral HPLC or other methods available in the art, such as diasteriomeric salt formation or a diol intermediate. *Id.* at 1266. Thus, the court concluded that the Smith reference was not enabiling prior art, because the resolution of the separate enantiomers of citalopram would have required undue experimentation based on the knowledge of one of ordinary skill in the art at the time. *Id.* 1267.

The fact pattern in *Forest Laboratories v. Ivax Pharmaceuticals* is similar to the instant case. The disclosure of Fire *et al.* merely mentions that the invention can be practiced in various cell types including vertebrate cells, but provides no direction on how to practice the invention in these various cell types. As documented above, the skilled artisan, notwithstanding the disclosure of Fire *et al.*, could not effectively practice the invention in vertebrate cells as several people in the field failed to effectively silence

target genes in vertebrate cells. Therefore, undue experimentation was required to apply the teachings of Fire *et al.* to vertebrate cells indicating that Fire *et al.* is not an enabling disclosure with respect to vertebrate cells.

In summary, appellants submit that U.S. Patent No. 6,506,559 to Fire *et al.* fails to anticipate claims 75, 76, 78, 79, 82-91, and 93-98 as Fire *et al.* does not provide an enabling disclosure for attenuating expression of a target gene in a vertebrate cell. The claims require that the target gene expression in the vertebrate cell is actually attenuated. As described above, at the time the present invention was made the field was unsure whether RNAi mechanisms even existed in vertebrate cells. Moreover, the skilled artisan was well aware of the non-specific stress response induced by administration of dsRNA to vertebrate cells that lead to a general suppression of protein synthesis and cell apoptosis. Therefore, one of ordinary skill in the art would not have believed the methods disclosed in Fire *et al.* would produce sequence-specific inhibition of gene expression in vertebrate cells because the administered dsRNA would be expected to activate mechanisms including the PKR kinase and inhibit all gene expression. Those that attempted to use methods similar to that disclosed in Fire *et al.* failed to achieve the desired end and observed the expected non-specific response known to be induced by dsRNA in vertebrate cells. Thus, given the state of the art and the knowledge of a skilled artisan at the time the invention was made, it was not within the ordinary skill in the art to attenuate gene expression of target genes in vertebrate cells despite the disclosure of Fire *et al.*

B. <u>Claim 78</u>

As noted above, the Examiner has finally rejected claim 78 under 35 U.S.C. 102(e) as being unpatentable over U.S. Patent No. 6,506,559 to Fire *et al.* Claim 78 requires the further limitation that the double stranded RNA supplied to the explanted vertebrate cell has a length of less than about 200 base pairs. Fire *et al.* do not teach using a double stranded RNA of less than about 200 base pairs. Although Fire *et al.* specify that the length of the nucleotide sequence identical to the target can be 25, 50, 100, 200, 300 or 400 bases (see column 8, lines 5-6), the specification is silent on the

total length of the double stranded RNA molecules that can be used in the claimed methods. All of the double stranded RNA molecules used in the working examples exceeded 200 base pairs with lengths ranging from 299 base pairs to 1033 base pairs. See Table 1 at column 22-column 23 of the Fire *et al.* specification. At no point during prosecution did the Examiner assert or demonstrate that Fire *et al.* teach a double stranded RNA of less than about 200 base pairs for attenuating target gene expression in an explanted vertebrate cell.

To anticipate a claim, a single source must contain all of the elements of the claim. See *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379, 231 USPQ 81, 90 (Fed. Cir. 1986); *Atlas Powder Co. v. E.I. du Pont De Nemours & Co.*, 750 F.2d 1569, 1574, 224 USPQ 409, 411 (Fed. Cir. 1984); *In re Marshall*, 578 F.2d 301, 304, 198 USPQ 344, 346 (CCPA 1978). Missing elements may not be supplied by the knowledge of one skilled in the art or the disclosure of another reference. See *Structural Rubber Prods. Co. v. Park Rubber Co.*, 749 F.2d 707, 716, 223 USPQ 1264, 1271 (Fed. Cir. 1984). Given that Fire *et al.* do not teach either explicitly or inherently using a double stranded RNA of less than about 200 base pairs in length, claim 78 is not anticipated by U.S. Patent No. 6,506,559 to Fire *et al.* Appellants submit that the rejection is improper and claim 78 is patentable over Fire *et al.*

II. Rejection under 35 U.S.C. 103(a) over U.S. Patent No. 6,506,559 in view of Ekenberg *et al.*

As explained previously, it appears that the rejection under 35 U.S.C. 103(a) is applied to claim 92 despite the Examiner's listing of all pending claims in the rejection. The Examiner's arguments supporting the rejection are only directed to the limitation of treating the double stranded RNA with RNase prior to delivery to the cell. The Examiner does not make any remarks as to why the invention as a whole is obvious over either U.S. Patent No. 6,506,559 or Ekenberg *et al.* singly or in combination. See pages 4-5 of Final Office Action dated December 22, 2006. Thus, the Examiner fails to set forth an alleged *prima facie* case of obviousness for all claims except claim 92. Therefore, appellants'

arguments are directed to the rejection of claim 92 under 35 U.S.C. 103(a) as being allegedly unpatentable over U.S. Patent No. 6,506,559 in view of Ekenberg *et al.*

One of ordinary skill in the art would not be motivated to modify the methods disclosed in Fire *et al.* with those disclosed in Ekenberg *et al.* or any other cited reference for application in vertebrate cells with a reasonable expectation of success. As described in detail above in the arguments against the rejection under 35 U.S.C. 102(e), the skilled artisan would not have believed the administration of double stranded RNA as disclosed by Fire *et al.* would have worked in vertebrate cells due to the well known stress response. Ekenberg *et al.* does not make up for the deficiencies of Fire *et al.*, therefore, the combination of Fire and Ekenberg does not render the claimed invention obvious.

With regard to the limitation of treating the double stranded RNA with RNase as recited in claim 92, the Examiner alleges that Fire *et al.* teach purification of RNA, but acknowledges that Fire *et al.* do not specifically teach purification with RNase prior to administration. The Examiner asserts that it was well known in the art at the time of the invention that RNases such as RNase A and RNase T degrade single stranded RNA specifically and cites Ekenberg *et al.*, which discloses a protocol for a RNase protection assay using these RNases. The Examiner concludes that it would have been obvious to one of ordinary skill in the art to modify the teachings of Fire *et al.* with those of Ekenberg *et al.* to purify the RNA using RNase, and such a skilled artisan would be motivated to do so since Fire *et al.* specifically suggest use of purified RNA. See pages 4 and 5 of Final Office Action dated December 22, 2006. Appellants disagree with the Examiner.

While Fire *et al.* do teach various methods to purify RNA, there is no disclosure that the purification can be enzymatic or that the purification encompasses separating double stranded RNA from single stranded RNA as would be the result of RNase treatment (see column 9, lines 16-21). In fact, Fire *et al.* state that "Alternatively, the RNA may be used with no or a minimum of purification to avoid losses due to sample processing" (column 9, lines 21-23). Thus, contrary to the Examiner's assertion, Fire *et al.* do not specifically recommend the use of purified RNA over non-purified RNA.

Moreover, use of RNase would be incompatible with the specific working examples in Fire *et al.*, which employed a mixture of sense and antisense strands.

Treatment of such mixtures of sense and antisense strands with RNases as disclosed by Ekenberg *et al.* could destroy the RNA molecules and render the invention unfit for its intended purpose. There is no suggestion or motivation to make the proposed modification, if the proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984). Thus, one of ordinary skill in the art would not be motivated to treat the RNA disclosed in Fire *et al.* with a RNase disclosed in Ekenberg *et al.* because the RNase would destroy the sense and antisense RNA molecules used by Fire *et al.* to administer double stranded RNA. Appellants submit that neither reference alone or taken together suggest treating double stranded RNA with RNase prior to delivery to an explanted vertebrate cell. Thus, claim 92 is nonobvious and patentable over the cited references.

Summary

In summary, the appellants submit that the invention is novel over U.S. Patent No. 6,506,559 to Fire *et al.* For the reasons detailed herein, Fire *et al.* do not provide an enabling disclosure of attenuating target gene expression in vertebrate cells and thus cannot anticipate the currently pending claims. Notwithstanding the publication of the disclosure of Fire *et al.*, the skilled artisan frequently experienced failures when attempting to achieve sequence-specific gene silencing in vertebrate cells by administering double stranded RNA due to the non-specific stress response, suggesting that Fire *et al.* did not place one of ordinary skill in the art in possession of the invention for practice in vertebrate cells. The appellants further submit that the invention is nonobvious and patentable over U.S. Patent No. 6,506,559 to Fire *et al.* in view of Ekenberg *et al.* Ekenberg *et al.* does not make up for the deficiencies of Fire *et al.*, and even if the teachings of the two references were combined, the claimed methods of Fire *et al.* would be inoperable as disclosed since Fire *et al.* employed a mixture of sense and antisense strands.

Accordingly, it is submitted that the Examiner's §§102(e) and 103(a) rejections of the claims are in error and should be reversed.

Respectfully submitted
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By: Sandy Truehart
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APPENDIX

CLAIMS ON APPEAL

75. A method for attenuating the expression of a target gene in a vertebrate cell *ex vivo* comprising:

explanting a vertebrate cell from a vertebrate organism;

supplying the cell with at least one double stranded RNA in an amount sufficient to specifically attenuate expression of the target gene, wherein one of the strands of the double stranded RNA is capable of hybridizing to the target gene *in vitro* in 400 mM NaCl, 40 mM PIPES ph 6.4, and 1 mM EDTA, at 50 °C; and

implanting the cell into a vertebrate organism, wherein expression of the target gene is attenuated in said vertebrate cell.

76. The method of claim 75, wherein the cell is implanted back into the vertebrate organism from which it was explanted.

78. The method of claim 75, wherein the double stranded RNA has a length of less than about 200 bases.

79. The method of claim 75, wherein the double stranded RNA comprises a nucleotide sequence that is complementary to a region of at least about 25 bases of the target gene.

82. The method of claim 75, wherein the double stranded RNA is supplied to the cell by delivery to the cell of the double stranded RNA.

83. The method of claim 82, wherein the double stranded RNA is purified in the absence of phenol or chloroform.

84. The method of claim 75, wherein the double stranded RNA is supplied to the cell by delivering to the cell a DNA encoding the double stranded RNA.

85. The method of claim 75, wherein the target gene is an endogenous gene.

86. The method of claim 75, wherein the target gene is a foreign gene.

87. The method of claim 75, wherein the target gene is a chromosomal gene.

88. The method of claim 75, wherein the target gene is an extrachromosomal gene.

89. The method of claim 75, wherein the double stranded RNA is supplied in an amount to completely inhibit expression of the target gene.

90. The method of claim 75, wherein the double stranded RNA comprises a single strand comprising self-complementary portions.

91. The method of claim 75, wherein the double stranded RNA comprises two separate complementary strands.

92. The method of claim 82, wherein the double stranded RNA is treated with Rnase prior to delivery to the cell.

93. The method of claim 91, wherein the two strands of the double stranded RNA are annealed in the presence of potassium chloride prior to delivery.

94. The method of claim 75, wherein the function of the target gene is unknown.

95. The method of claim 75, further comprising identifying a phenotypic change in the vertebrate cell associated with attenuated expression of the target gene.

96. The method of claim 75, wherein the target gene is associated with a disease.

97. The method of claim 75, wherein the target gene is associated with a pathogen.

98. The method of claim 97, wherein the pathogen is selected from the group consisting of a virus, bacterium, fungus or protozoan.

EVIDENCE APPENDIX

Exhibit A- U.S. Patent No. 6,506,559 to Fire *et al*

Exhibit B- Isaacs and Lindenmann (1957) Proc. R. Soc. Lond. (Biol) 147: 258-267

Exhibit C-Carter and DeClercq (1974) Science 186: 1172-1178

Exhibit D- Clemens and Elia (1997) J. Interferon Cytokine Res. 17: 503-524

Exhibit E- Proud, C.G. (1995) Trends in Biochemical Sciences 20: 241-246

Exhibit F- Manche *et al.* (1992) Mol. Cell. Bio. 12: 5238-5248

Exhibit G- Sioud, M. (2005) J. Mol. Bio. 348: 1079-1090

Exhibit H- Reynolds et al. (2006) RNA 12: 1-6

Exhibit I- Tuschl *et al.* (1999) Genes and Development 13: 3191-3197

Exhibit J- Caplen *et al.* (2000) Gene 252: 95-105

Exhibit K- Oates *et al.* (2000) Developmental Biology 224: 20-28

Exhibit L- Caplen *et al.* (2001) Proc. Natl. Acad. Sci. USA 98: 9742-9747

Exhibit M- Elbashir *et al.* (2001) Nature 411: 494-498

Exhibit N- Montgomery and Fire (1998) Trends in Genetics 14: 255-258

Exhibit O- *Impax Laboratories, Inc. v. Aventis Pharmaceuticals, Inc.*, 496 F. Supp. 2d 428 (D. Del. 2007)

Exhibit P- *Forest Laboratories Inc. v. Ivax Pharmaceuticals, Inc.*, 501 F.3d 1263, (Fed. Cir. 2007)

RELATED PROCEEDINGS APPENDIX

None